Chronic lymphocytic leukemia – aiming at a moving target!

Peter Hillmen
Department of Hematology, Leeds General Infirmary, Great George Street, Leeds, LS1 3EX United Kingdom.
E-mail: peter.hillmen@nhs.net

Over the last 5 to 10 years there have been dramatic changes in our understanding of the biology of chronic lymphocytic leukemia (CLL). Until recently CLL was generally considered to be a single disease entity with little biological heterogeneity. It was thought to result from the expansion of terminally differentiated mature B-lymphocytes with a low proliferative rate and defective apoptosis. It is now clear that this perception is ill-conceived. Several independent lines of evidence have converged to radically alter our perception of the disease.

Experiments with deuterated water (^2H_2O) demonstrate that the CLL clone is proliferating at an appreciably higher rate than previously thought (0.1 to >1.0% of the cells being *born* each day).[1] Indirect assumptions from this work indicate that there must be relatively rapid apoptosis of the CLL clone, otherwise there would be a rapid increase in the number of CLL cells and rapid progression of the disease. There is compelling evidence that the *proliferation centers* found in the bone marrow and lymph nodes of most patients are the sites where most proliferation of CLL cells occur.[2] However a specific immunophenotype of the proliferating compartment has not been identified. This might be because the cells are continually cycling into and out of the areas of proliferation and, as has been previously described, are getting transient survival signals preventing them from undergoing apoptosis.

It is apparent that the CLL cells of many patients preferentially utilize similar variable immunoglobulin genes. In particular, V_H3-21 and V_H1-69 are more commonly utilized than would be predicted by chance.[3] Some patients use identical entire VDJ (variable-diversity-joining) segments whereas some patients even utilize the same light chain genes. For example, many patients using V_H3-21 also use V_λ2-14 more frequently than expected.[4] The probability of this occurring by chance are virtually zero. These observations strongly suggest that the B-cell receptor of the CLL clone is specific for a common antigen, in at least a proportion of patients. The antigen has not yet been identified but probably has a role in the development of the CLL clone.

The observation that patients in whom the immunoglobulin gene of the CLL clone had been subjected to the normal physiological process of somatic mutation have a good prognosis whereas patients without somatic mutations (germ-line sequence) have a poor prognosis is pivotal to our understanding of CLL. This observation, first described by two groups in 1999,[5,6] has been confirmed by a number of investigators and collaborative groups. The first question to consider is whether these two entities are different disorders with a similar immunophenotype or whether they are two variations of the same disease. cDNA microarray analysis comparing cells from mutated and unmutated CLL demonstrate that the two forms of the disease are more similar to each other than to other chronic lymphoproliferative disorders or any normal B-cell subset.[7]

At present in order to reliably differentiate cases of mutated from unmutated CLL it is necessary to sequence the variable heavy chain (V_H) and then to compare this to the most homologous germ-line V_H gene. If the sequence has less than 2% variation from the germ-line sequence then it is considered unmutated and therefore identifies a patient with poor risk disease. Conversely, cases with less than 98% homology to germ-line are considered mutated and good risk. To perform this as a routine test for all patients would not be a trivial exercise. The cDNA microarray experiments have identified a small number of genes that are differentially expressed by mutated and unmutated CLL. It is possible that these differentially expressed genes could be used to differentiate good risk and poor risk CLL more easily. One such molecule is zeta-associated protein 70 (ZAP-70),[8] an intracellular signaling molecule normally expressed in T cells but only very rarely in normal B cells, is a molecule that has been identified through microarray analysis.

Syk is both structurally and functionally homologous to ZAP-70 but is expressed in normal B cells. ZAP-70 is pivotal in the transmission of signals from the T-cell receptor to the nucleus whereas Syk transmits signals from the B-cell receptor (BCR) in normal B cells. Thus the expression of ZAP-70 in CLL cells is aberrant but there is evidence that it may be replacing or augmenting the function of Syk in BCR signaling. Several studies have correlated ZAP-70 expression with mutational status and prognosis.[9-11] Therefore ZAP-70 is potentially a surrogate for mutational status but the analysis of ZAP-70 is not straightforward resulting in many different analytical approaches being reported, including immunohistochemistry, flow cytometry, western and northern blotting.

The flow cytometric approach is technically difficult to perform mainly because the expression of ZAP-70 in B-CLL cells is weak and unimodal making the cut-off for positivity difficult to standardize. Therefore a patient may be called ZAP-70 positive in one laboratory but negative in another. This has led to the initiation of an International Project under the auspices of ERIC (European Research Initiative on CLL) and the LeukemiaNet designed to standardize the testing for ZAP-70.

In this issue of the journal, Laurenti *et al*. (see page 1533) describe the use of polymerase chain reaction to assess the level of ZAP-70. The unique feature of their technique is the utilization of the level of expression of Syk in the same CLL cells as a comparator for ZAP-70 expression. They have used the ratio of ZAP-70 to Syk mRNA to define the status of individual patients. A ratio of greater than 0.25 was observed in patients with a short treatment-free survival and often unmutated CLL whereas absent or negligible ZAP-70 (ratio <0.05) was associated with a better outcome and a mutated genotype. If confirmed by other groups this technique might allow for a clearer, more reliable separation of good risk from poor risk disease and hence could facilitate the planned trials which will test whether good risk patients should be treated with a different regimen from that used for poor

risk patients.

To add to the complexity of the disease, CLL clones are not genetically stable as they frequently develop recurrent additional chromosomal aberrations which can be identified by interphase fluorescence *in situ* hybridization in approximately 80% of patients.[12] The loss of the short arm of chromosome 17 (17p-), the location of the p53 oncogene, and the loss of the long arm of chromosome 11 (11q-), most probably the location of the ataxia telangiectasia mutated (ATM) gene, are both associated with a poor response to therapy and worse survival. Both these abnormalities are more frequent in previously treated and refractory patients with CLL and confer resistance to conventional chemotherapies. Thus CLL is a biologically heterogeneous disorder, with actively proliferating cells, which at least in some cases is antigen driven at some stage in the development of the disease and can be genetically unstable with the development of sub-clones with chemotherapy-resistant genotypes (*see below*).

The rapid progress in our understanding of CLL and the description of novel prognostic indicators that reliably define the outcome for individual patients appears to have created more general uncertainty about how to treat CLL. This is in part because the initial reports of these novel factors in patients with CLL has emanated from the analysis of large retrospective series of patients without prospective data being available. The rapid development and diversity of novel prognostic markers in CLL has created considerable uncertainty regarding the relative importance of each marker and has left many clinicians bewildered as to how to incorporate this new information into the treatment paradigm of CLL. Information from prospective therapeutic trials is only now beginning to emerge and, as yet, no trial has used the newer markers to define therapeutic approaches.

The description of the new biological markers raises important questions and creates several clinical opportunities. Firstly, in stage A CLL such analysis should allow for a more accurate prediction of which patients will remain stable and which are more likely to progress rapidly to require therapy – this is often the first question that any patient asks after the diagnosis of CLL has been established. Studies are currently being planned to test the hypothesis that *poor risk* stage A patients should be treated before disease progression but there is as yet no evidence at all that early intervention in these patients will have a positive impact on overall survival and, in fact it is plausible that these patients will have worse survival if exposed to potentially toxic chemotherapy earlier than is currently practised – these patients have genetically unstable disease and exposure to mutagenic chemotherapy may induce mutations in tumor suppressor genes, oncogenes and DNA repair genes resulting in a more resistant disease. Therefore it is essential that stage A patients should only be treated if they are included in well designed clinical trials.

The second key question is whether patients with good risk disease should be treated with less intensive therapy and whether high risk patients should be offered more intensive therapy, and even reduced intensity conditioning allogeneic transplants if they have a suitable donor. The eradication of minimal residual disease to below detectable levels may well confer a survival advantage[13] but this also needs to be confirmed in well-designed randomized controlled trails.

The next generation of clinical trials are currently being designed to incorporate the molecular markers into the therapeutic pathway for CLL but it will be some years before such prospective data are available to influence the treatment of individual CLL patients. Thus at present it is far too early to use this information to make potentially critical treatment decisions.

Acknowledgments: the author would like to acknowledge Dr. Andy Rawstron for his critical review of the manuscript.

References

1. Messmer BT, Messmer D, Allen SL, Kolitz JE, Kudalkar P, Cesar D, et al. In vivo measurements document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. J Clin Invest 2005; 115:755-64.
2. Stevenson F, Caligaris-Cappio F. Chronic lymphocytic leukemia: revelations from the B-cell receptor. Blood 2004;103: 4389-95.
3. Johnson TA, Rassenti LZ, Kipps TJ. Ig VH1 genes expressed in B cell chronic lymphocytic leukemia exhibit distinctive molecular features. J Immunol 1997;158:235-46.
4. Falt S, Merup M, Tobin G, Thunberg U, Gahrton G, Rosenquist R, et al. Distinctive gene expression pattern in VH3-21 utilizing B-cell chronic lymphocytic leukemia. Blood 2005; 106:681-9.
5. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999; 94:1848-54.
6. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. IgV gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94:1840-7.
7. Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001;194:1639-47.
8. Wiestner A, Rosenwald A, Barry TS, Wright G, Davis RE, Henrickson SE, et al. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood 2003;101:4944-51.
9. Crespo M, Bosch F, Villamor N, Bellosillo B, Colomer D, Rozman M, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. N Engl J Med 2003;348:1764-75.
10. Orchard JA, Ibbotson RE, Davis Z, Wiestner A, Rosenwald A, Thomas PW, et al. ZAP-70 expression and prognosis in chronic lymphocytic leukaemia. Lancet 2004;363:105-11.
11. Rassenti LZ, Huynh L, Toy TL, Chen L, Keating MJ, Gribben JG, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N Engl J Med 2005; 351:893-901.
12. Döhner H, Stilgenbauer S, Benner A, Leupolt E, Kröber A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343:1910-6.
13. Moreton P, Kennedy DB, Lucas G, Leach M, Rassam SMB, Haynes A, et al. Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. J Clin Oncol 2005; 23:2971-9.